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PROKINETIC AND LAXATIVE EFFECTS OF XIAO'ER QIXINGCHA, A HOUSEHOLD PEDIATRIC HERBAL FORMULA

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Abstract

Background: Xiao'er Qixingcha, a household Chinese Medicinal formula, has been extensively applied in pediatric clinic for dyspepsia and constipation for hundreds of years. The present study firstly inspected whether the extract of Xiao'er Qixingcha (EXQ) has *in vivo* and *in vitro* prokinetic and laxative effects, and evaluated its acute toxicity.

Materials and Methods: In the *in vivo* study, small intestinal transit rates and fecal output characters (fecal number and fecal weight) were measured on normal and two models of constipated mice (induced by diphenoxylate and by water-fasting respectively). In the *in vivo* study, the contraction rates of ileum smooth muscle were examined with EXQ treatment. Moreover, in acute toxicity study, EXQ was administered orally for 14 days to juvenile SD rats, and clinical signs, viscera lesion and body weight were monitored daily.

Results: EXQ at all doses significantly increased the small intestinal transit rates, and ameliorated the fecal output characters of normal mice. In diphenoxylate-induced constipated mice, EXQ dose-dependently improved the small intestinal transit rates and fecal output. In water-fasting-induced constipated mice, EXQ dose-dependently improved the small intestinal transit rates, and significantly ameliorated the fecal output characters at 2.92 and 6.75 g/kg. Furthermore, in the *in vitro* study, EXQ dose-dependently raised the contraction rates of the isolated rabbit ileum smooth muscle. Finally, the acute toxicity study indicated that no toxicological effect was observed in terms of clinical signs, viscera lesion or change of body weight.

Conclusions: Taken together, EXQ exhibited prominent prokinetic and laxative activities, promising it as a safe and effective alternative pharmaceutical therapy for constipation.

Key words: Xiao'er Qixingcha, Constipation, Prokinetic, Laxative, Diphenoxylate, Water-fasted.

Introduction

Xiao'er Qixingcha is a well-known pediatric herbal medicine in Hong Kong and China, and has been extensively applied to relieve dyspepsia and constipation of infants for hundreds of years. As embodied in Pharmacopoeia of People's Republic of China (Pharmacopoeia of PR China, 2010), Xiao'er Qixingcha consists of seven herbs, which are *Semen coicis*, *Fructus oryzae germinatus*, *Fructus crataegi*, *Lophatherum gracile*, *Uncaria rhynchophylla*, *Cicada slough* and *Glycyrrhiza uralensis*. Its therapeutic functions in Chinese medicine are to improve appetite, relieve dyspepsia, clear heat and arrest convulsion. Clinically, Xiao'er Qixingcha can relieve discomfort due to indigestion, dyspepsia, poor appetite, dysphoria, restless sleep, constipation and dysuria (Pharmacopoeia of PR China, 2010). It has been developed into various preparations (such as

sirup, granula and oral liquid, etc.) which are available over the counter year-around. However, despite the extensive application of Xiao'er Qixingcha, few information of its pharmacological effect is available.

Constipation, defined as infrequent or difficult defecation, is one of the most common dysfunctional gastrointestinal symptoms in both developed and developing countries (Zhou et al., 2013). Constipation occurs in up to 27% of the general population and affects all age groups (Longstreth et al., 2006). It not only causes physical and mental suffering for patients but also has adverse effects on their quality of life by increasing the risk of anxiety, abdominal distension, vomiting, gut obstruction and perforation (Mostafa et al., 2003). To date, a lot of drugs, including bulking agents, stool softeners, osmotic laxatives and stimulant laxatives, have been used cautiously for treating constipation (Liu, 2011, Wald, 2007). However, their adverse effects, such as nausea, vomit, abdominal pain, dependency and cathartic colon, have raised public concern (Liu, 2011, Wald, 2007). Hence, the search for safe and effective laxative never stops in consideration of various harmful adverse effects and the prevalence of constipation.

Therefore, the present investigation aims to evaluate the prokinetic and laxative effects of EXQ and its acute toxicological properties for the first time. In the *in vivo* study, we assessed its effects on small intestinal transit and fecal output character in normal and two models of experimental constipated mice (induced either by diphenoxylate or by water-fasting). In the *in vitro* study, we examined its prokinetic effects on smooth muscle contraction of rabbit ileum.

Materials and Methods

Animals

Kunming mice (weighed 18-22 g), SD rats (weighted 50-70g) and New Zealand rabbits (weighed 2.0-2.5kg) were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine (Approval number SCXK (Guangzhou)-2013-0020). The animals were housed under standard environment conditions (22±1℃, humidity 50-70%, 12 h light: 12 h dark cycle) with free access to standard diet and water *ad libitum*. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animal of the National Institute of Health as well as Guide of the Animal Welfare Act.

Materials

The prescription of Xiao'er Qixingcha is shown in Table 1. EXQ was provided by Guangzhou Wanglaoji pharmaceutical company limited (Batch No. 201302). The extraction process was performed in accordance with the Chinese Pharmacopoeia (Pharmacopoeia of PR China, 2010). Briefly, *Semen coicis* and *Fructus oryzae germinates* were extracted with boiling water twice, two hour for each time. After filtration, the aqueous extract was concentrated to a relative density of 1.08-1.12 by rotary evaporator under vacuum. Ethanol was added into the concentrated extract until the percentage of ethanol was amounted to 45%, and then stored at 4℃.The obtained extract was centrifuged for 15 min at 3000 rpm and the supernatant was concentrated to obtain thick paste. The rest herbs were extracted with distilled water at 100℃ twice, two hour for each time. The resulting extract was filtered and concentrated to an appropriate amount, and then blended with the aforementioned thick paste. The extraction yield was 13.57%, i.e.1g of the extract equaled to 7.3693g crude material.

Table 1: Prescription of Xiao'er Qixingcha

Herbs	Weight ratio (%)
Semen coicis	25.02
Fructus oryzae germinatus	25.02
Fructus crataegi	13.30
Lophatherum gracile	19.98
Uncaria rhynchophylla	10.00
Cicada slough	3.34
Glycyrrhiza uralensis	3.34

Small Intestinal Transit Rates in Normal Mice

The method to evaluate the prokinetic and laxative effects on small intestinal transit and fecal output character in normal and two models of experimental constipated mice were based on previous reports (Xu et al., 2012, Zhou et al., 2013, Shan et al., 2010). Briefly, fifty animals were randomly divided into five groups (5 male and 5 female each group): normal group, Neosigmine Methylsuifate group (NM), and three doses of EXQ groups (2.92, 4.38 and 6.75 g/kg). EXQ groups were treated with EXQ (2.92, 4.38 and 6.75 g/kg, i.g.) while normal and NM groups were treated with saline (1.5 ml/10g, i.g.) for 6 consecutive days, respectively. At the 7th day, saline and EXQ were all prepared with distilled water suspension containing 5% charcoal and 10% gum acacia. After fasting for 16 h with free access to water, mice in normal group received saline; NM group was administered with Neosigmine Methylsuifate (0.2 mg/kg, i.p. A positive agent for screening and evaluating potential prokinetic and laxative agents) and saline (1.5 ml/10g, i.g.), and EXQ groups were treated with three doses of EXQ respectively. The mice were killed by cervical dislocation 30 min after charcoal meal administration. The small intestine from the pylorus to the caecum was quickly removed and the distance traveled by the charcoal meal and the total length of the intestine were measured. The small intestinal transit rate was calculated as the percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine.

Fecal Output Character in Normal Mice

Mice (16 male and 16 female) were randomly divided into four groups (n=8 in each group): normal group and three doses of EXQ groups (2.92, 4.38 and 6.75 g/kg). Administration of these four groups was the same as aforementioned in “Small intestinal transit rates in normal mice”. Mice were immediately placed in small transparent cages individually after administration with charcoal meal at the 7th day. Feces in 6 h after administration were collected, counted and weighed.

Small Intestinal Transit Rates in Diphenoxylate-Induced Constipated Mice

Sixty mice were randomly divided into six groups (5 male and 5 female each): normal group, diphenoxylate group (DC), Neosigmine Methylsuifate group (NM) and EXQ groups (2.92, 4.38 and 6.75 g/kg). Normal, DC and NM groups were given saline (1.5 ml/10g, i.g.) and EXQ groups were treated with EXQ (2.92, 4 38 and 6.75 g/kg, i.g.) for 6 consecutive days, respectively. At the 7th day, saline and EXQ were all prepared with distilled water suspension containing 5% charcoal and 10% gum acacia. After fasting for 16 h with free access to water, mice in normal group were administered with normal saline, while the other animals were treated with diphenoxylate (50 mg/kg, i.g) which is a constipation inducer. Thirty minutes later, mice in normal and DC groups received saline (1.5 ml/10g, i.g.); NM group was administered with Neosigmine Methylsuifate (0.2 mg/kg, i.p.) and saline (1.5 ml/10g, i.g.); and EXQ groups were treated with three doses of EXQ respectively. Experimental processes were performed as aforementioned in “Small intestinal transit rates in normal mice”.

Fecal Output Character in Diphenoxylate-Induced Constipated Mice

Animal grouping and administration were in accordance with “Small intestinal transit rates in diphenoxylate-induced constipated mice”. All experimental procedures were performed in line with “Fecal output character in normal mice”.

Small Intestinal Transit Rates in Water-Fasted-Induced Constipated Mice

Fifty mice (25 male and 25 female) were randomly divided into five groups (n = 10 in each group): water-fasted group (WF), Neosigmine Methylsuifate group (NM) and EXQ groups (2.92, 4.38 and 6.75 g/kg). WF group received saline (1.5 ml/10g, i.g.) and other groups were treated as described in “Small intestinal transit rates in diphenoxylate-induced constipated mice” for 6 consecutive days. Mice were access to diet *ad libitum* but fasted water for 72 h to induce constipation. At the 7th day, saline and EXQ were all prepared with distilled water suspension containing 5% charcoal and 10% gum acacia. Mice in WF group were served with saline and others were administered in accordance with section “Small intestinal transit rates in diphenoxylate-induced constipated mice”. Experimental processes were carried out as depicted in “Small

intestinal transit rates in normal mice".

Fecal Output Character in Water-Fasted-Induced Constipated Mice

Sixty mice (30 male and 30 female) were randomly divided into six groups (n=10 in each group): normal group, water-fasted group (WF), Neosigmine Methylsuifate group (NM) and three EXQ groups (2.92, 4.38 and 6.75 g/kg). Animal administration was in accordance with section "Small intestinal transit rates in water-fasted-induced constipated mice". All experimental procedures were performed in accordance with "Fecal output character in normal mice".

Contraction of Ileum Smooth Muscle *in Vitro*

The rabbits were euthanatized by air embolism, and the ileum was harvested quickly. The content of the ileum was flushed with Tyrode's solution and then cut into several segments, each approximately 1-cm in length. Each segment was longitudinally placed in a 10-ml vertical organ bath which was continuously perfused with Tyrode's solution (137 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM $MgCl_2 \cdot 6H_2O$, 0.3 mM $NaHPO_4 \cdot 2H_2O$, and 5.6 mM glucose; pH 7.4). The organ bath was bubbled with a mixture of 95% O_2 plus 5% CO_2, and maintained at 37 ℃. The mechanical activity of longitudinal smooth muscle was recorded using the PowerLab system and Chart 5 software (AD instrument Ltd., Australia) via an isotonic transducer. The tissues were stabilized for at least 20 min before the initiation of the experiment and the basal curve was obtained. Distill water (0 mg/ml) and EXQ at final concentrations of 1 mg/ml、2 mg/ml、4 mg/ml was added after the stabilization. All the tissues were washed three times after each response and left for at least 20 min for recovering spontaneous activity before adding another drugs. The area under the curve (AUC) of contractile response was measured before and after the addition of agents. The contraction effects were expressed as contraction rate which was quantified according to the equation: contraction rate = (the AUC after the addition of agents - the AUC before the addition of agents) / the AUC before the addition of agents.

Acute Toxicity Test

EXQ of 66.6g/kg (three times a day, 22g/kg for each) was administrated orally to the juvenile SD rats (10 male and 10 female in each group) for 14 days. The control group was given 20 ml/kg vehicle. In this period, animals were monitored daily for mortality, abnormal behavior and other toxic signs, and the body weight was recorded at day 1, 4, 7, 14 and 15. On day 15, all surviving animal were sacrificed and then autopsied and examined macroscopically for any pathological changes compared with the control group.

Statistical Analysis

All values were expressed as means ± SEM. The measurement data were analyzed by one-way ANOVA followed by the Dunnett t test. All statistical analyses were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL) and differences between means were considered statistically significant when $p < 0.05$ and $p < 0.01$.

Results

Small Intestinal Transit Rates in Normal Mice

The effect of EXQ on small intestinal transit rates in normal mice was shown in Figure 1. Compared with normal group (29.48±3.04%), treatment with Neosigmine Methylsuifate (40.85±2.35%, $p < 0.05$) and EXQ of three doses (44.78±3.90%, $p < 0.01$; 41.53±3.99%, $p < 0.05$; and 48.00±4.16%, $p < 0.01$, for 2.92, 4.38 and 6.75 g/kg respectively) significantly increased the small intestinal transit rates. The percentages of promotion on small intestinal transit rates were 38.58%, 51.89%, 40.87% and 62.82% for the groups treated with Neosigmine Methylsuifate and three doses of EXQ, respectively.

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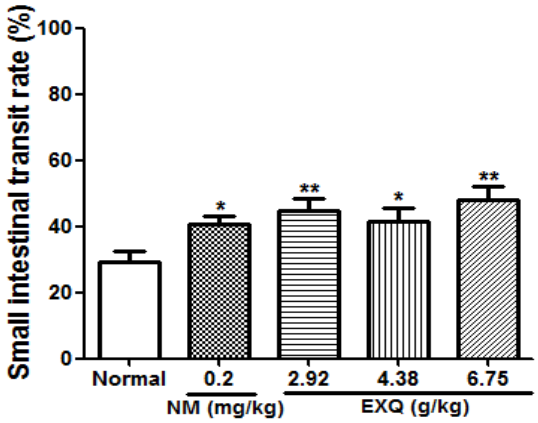

Figure 1: Effect of EXQ on small intestinal transit rates in normal mice.

Data express the means ± SEM (n=10). *p < 0.05 and **p < 0.01 compared with normal group. NM, Neosigmine Methylsuifate group (served as positive control).

Fecal Output Character in Normal Mice

Fecal number and fecal weight were determined in normal mice (Figure 2). When compared with the normal group (number: 5.26±0.53; weight: 61.99±5.89), mice treated with EXQ (2.92, 4.38 and 6.75 g/kg) exhibited significant increments in number (8.00±0.53, p < 0.01; 11.75±0.45, p < 0.01; 7.62±0.50, p < 0.05, respectively) and weight (87.16±7.30, p < 0.05; 153.98±5.92, p < 0.01; 128.54±8.42, p < 0.01, respectively) of feces. EXQ increased the fecal number by 35.56%, 108.89%, 42.22%, and fecal weight by 107.36%, 148.34%, 40.61%, in parallel to normal control.

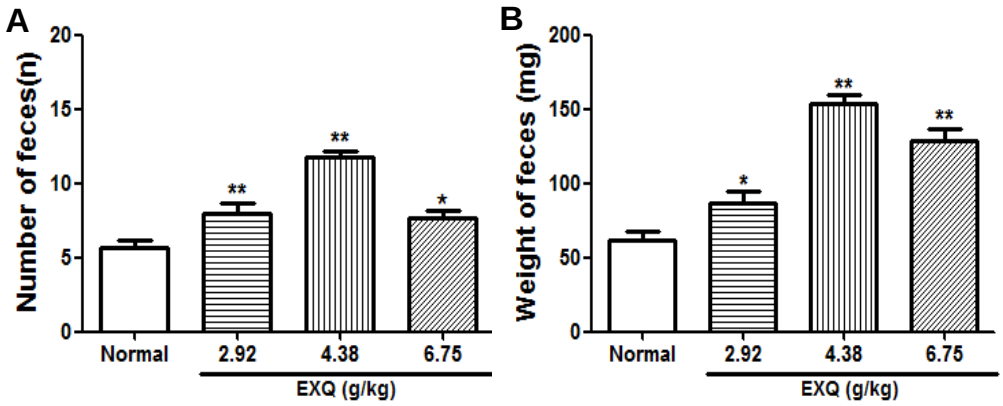

Figure 2: Effect of EXQ on fecal output character in normal mice. A: number of feces; B: weight of feces

Data express the means ± SEM (n=8). *p < 0.05 and **p < 0.01 compared with normal group.

Small Intestinal Transit Rates in Diphenoxylate-Induced Constipated Mice

Figure 3 indicates that diphenoxylate administration resulted in a significant inhibition in the small intestinal transit rates (38.04±2.91%, p < 0.01), compared with normal (78.08±4.31%). Neosigmine Methylsuifate offered marked elevation in small intestinal transit rate (87.13±3.78, p < 0.01 vs. Diphenoxylate group). Consistently, EXQ significantly increased the small intestinal transit rates in a dose dependent manner (65.63±5.13, 75.01±4.35, 90.74±3.23, for all p < 0.01 vs. Diphenoxylate group), presenting ascending amplitude of 72.51%, 97.19% and 138.54% over the diphenoxylate group, respectively.

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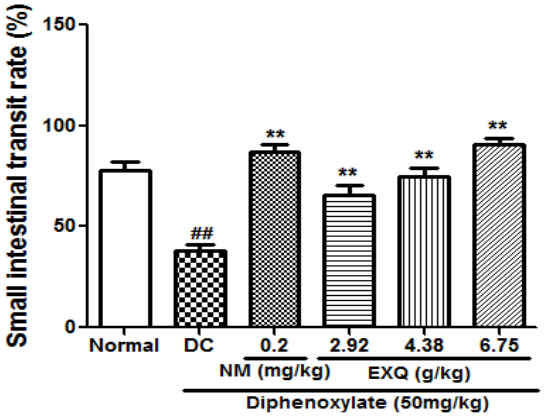

Figure 3: Effect of EXQ on small intestinal transit rates in diphenoxylate-induced constipated mice.

Data express the means ± SEM (n=10). $^{\#\#}p < 0.01$ compared with normal group, $^{**}p < 0.01$ compared with DC group. DC, diphenoxylate group (served as constipated control). NM, Neosigmine Methylsuifate group (served as positive control).

Fecal Output Character in Diphenoxylate-Induced Constipated Mice

As displayed in Figure 4, compared with the normal group (number: 10.20±0.49; weight: 112.13±5.37), diphenoxylate group showed significantly lower number (1.60±0.22, $p < 0.01$) and weight (19.78±2.62, $p < 0.01$) of fecal output, indicating that diphenoxylate reduced the fecal number and fecal weight to 84.31% and 82.56% of normal animals. Conversely, EXQ counteracted the decreases induced by diphenoxylate, in a dose-dependent manner. Compared with the diphenoxylate group, there was a prominent restoration of fecal number in EXQ groups at 4.38 and 6.75 g/kg (3.10±0.35, $p < 0.05$; 4.70±0.37, $p < 0.01$) and fecal weight in all doses of EXQ groups (47.81±7.34, 48.95±5.65, 64.29±4.78, for all $p < 0.01$), which means augmentations of 93.75% and 193.75% in fecal number, and increases of 141.71%, 147.47% and 225.02% in fecal weight. Likewise, significant effect was observed at Neosigmine Methylsuifate group both in fecal number (2.7±1.49, $p < 0.05$ vs. Diphenoxylate group) and fecal weight (31.79±17.58, $p < 0.05$ vs. Diphenoxylate group).

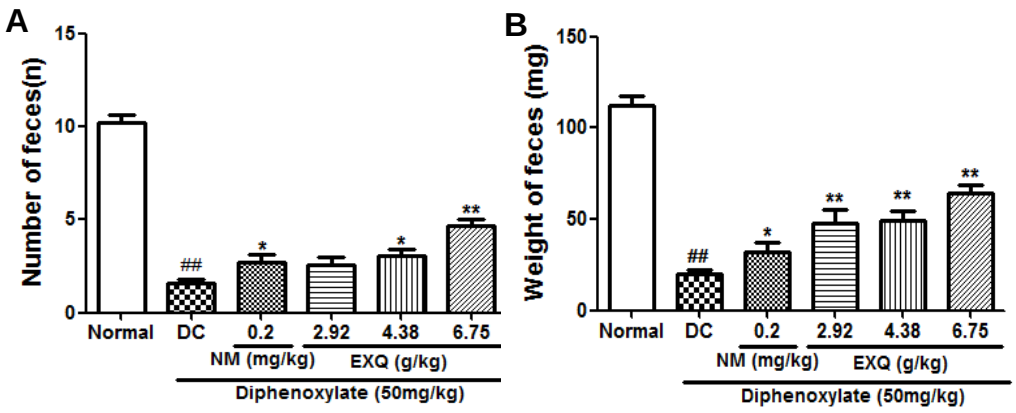

Figure 4: Effect of EXQ on fecal output character in diphenoxylate-induced constipated mice. A: number of feces; B: weight of feces

Data express the means ± SEM (n=10). $^{\#\#}p < 0.01$ compared with normal group, $^{**}p < 0.01$ compared with DC group. DC, diphenoxylate group (served as constipated control). NM, Neosigmine Methylsuifate group (served as positive control).

Small Intestinal Transit Rates in Water-Fasted-Induced Constipated Mice

To verify the prokinetic and laxative effects of EXQ against hydropenic condition, water-fasted-induced model was employed (Figure 5). In three EXQ groups (2.92, 4.38 and 6.75 g/kg), the propulsion rates of charcoal meal were 90.31±2.41%, 94.31±2.40% and 100.00±0.00% respectively, which means dose-related improvements of 56.41%, 63.79% and 73.66% (for all $p < 0.01$), as compared with the 57.58±4.41% of

water-fasted group. Neosigmine Methylsuifate group also significantly increased the small intestinal transit rates (82.22±3.80%, $p < 0.01$ vs. Water-fasted group).

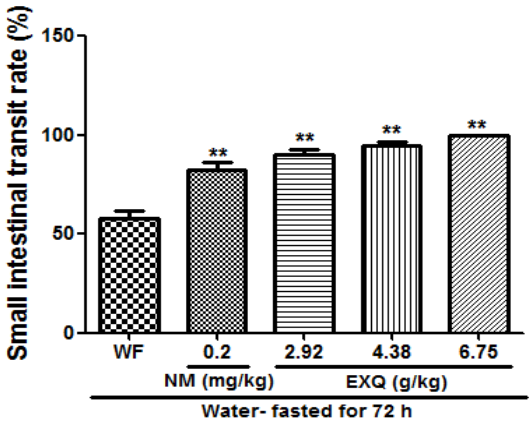

Figure 5: Effect of EXQ on small intestinal transit rates in water-fasted-induced constipated mice.

Data express the means ± SEM (n=10). **$p < 0.01$ compared with WF group. WF, Water-fasted group (served as constipated control). NM, Neosigmine Methylsuifate group (served as positive control).

Fecal Output Character in Water-Fasted-Induced Constipated Mice

After being fasted for 72 h, the mice had obvious hydropenic constipation (Figure 6). In contrast to the normal mice (number: 14.5±0.72; weight: 172.22±8.84), the number (3.50±0.45, $p < 0.01$) and weight (40.25±5.38, $p < 0.01$) of feces in water-fasted group were markedly lessened within 6 h after administration. Although the fecal number and fecal weight of all the three EXQ groups were still less than those of normal group, the EXQ groups at 2.92 and 6.75 g/kg had significantly increased number (5.80±0.44, $p < 0.01$; 5.60±0.45, $p < 0.05$) and weight (99.03±7.45, $p < 0.01$; 78.47±6.01, $p < 0.01$) of feces over the water-fasted group. It can be concluded that EXQ at doses of 2.92 and 6.75 g/kg elevated fecal number by 65.71% and 60.00%, and increased fecal weight by 146.34% and 90.96%. Likewise, Neosigmine Methylsuifate group markedly increased the fecal number (5.70±0.42, $p < 0.01$ vs. Water-fasted group) and fecal weight (65.55±4.97, $p < 0.05$ vs. Water-fasted group).

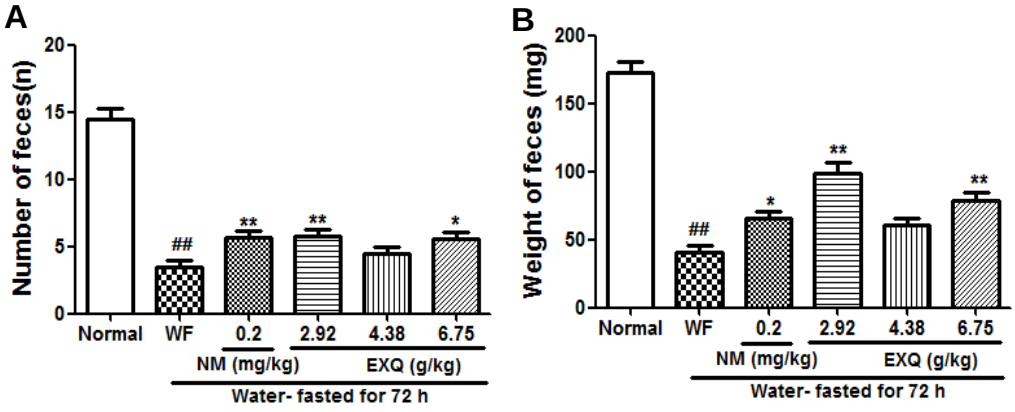

Figure 6: Effect of EXQ on fecal output character in water-fasted-induced constipated mice. A: number of feces; B: weight of feces

Data express the means ± SEM (n=10). ##$p < 0.01$ compared with normal group, *$p < 0.05$, **$p < 0.01$ compared with WF group. WF, Water-fasted group (served as constipated control). NM, Neosigmine Methylsuifate group (served as positive control).

Contraction of Ileum Smooth Muscle *in Vitro*

In addition, we evaluated the *in vitro* prokinetic effect of EXQ on contraction potency of ileum smooth muscle (Figure 7). EXQ was

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dose-dependently promoted the smooth muscle contraction of rabbit ileum. The contraction rates after EXQ treatment at the final concentrations of 2 mg/ml and 4 mg/ml were 45.59±7.91% and 60.75±8.68% (for both, $p < 0.01$), significantly increased when compared to that of 0 mg/ml (8.68±3.10%).

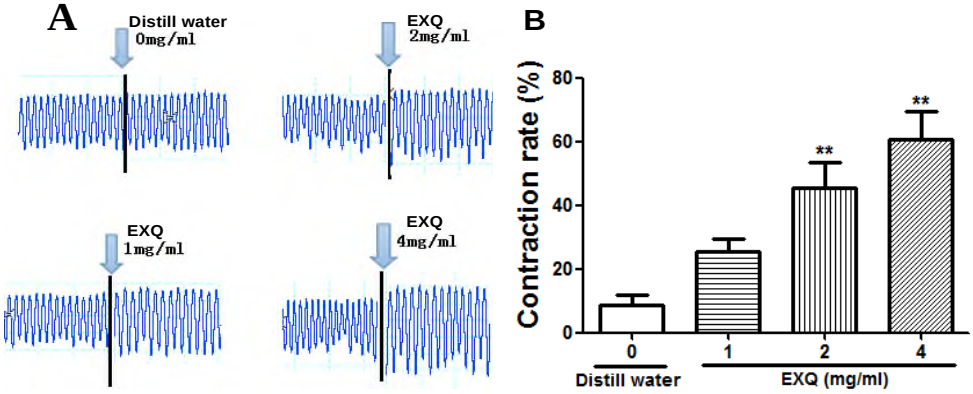

Figure 7: Effect of EXQ on rabbit ileum smooth muscle contraction *in vitro*. A: representative figure of ileum contraction after EXQ treatments; B: contraction rates

Data express the means ± SEM (n=10). **$p < 0.0$ 1 compared with 0mg/ml.

Acute Toxicity Evaluation

EXQ up to 66.6 g/kg/day (202 times of the clinical dosage) did not cause any deaths during the 14-day treatment, in which no change was observed in appearance, behavior, body weight ($p > 0.05$, Figure 8) or consumption of water and food. Moreover, autopsy showed no macroscopic morphology change of heart, lung, liver and kidney compared with the control group.

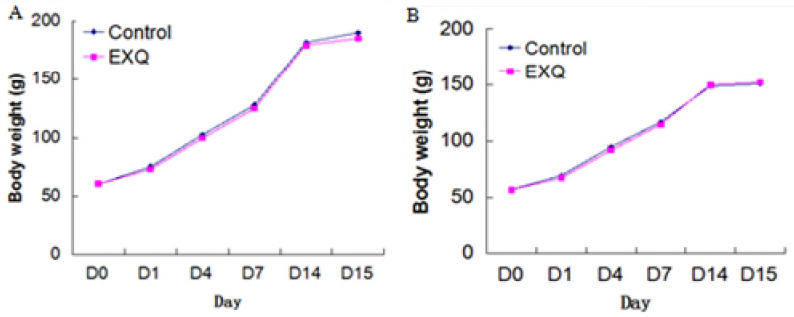

Figure 8: Effect of EXQ supplementation on the changes of body weight. A: male; B: female

Discussion

Constipation is a public health problem with a high prevalence which seriously affects the quality of life. However, the existing treatments for constipation are still far from satisfactory due to various adverse effects (Liu, 2011, Wald, 2007). Currently, active researches have rekindled interest in natural drugs, such as *Liriope platyphylla* (Kim et al., 2013) and agarwood (Hara et al., 2008), and there has been an increased inclination towards herbal formulations like Ma Zi Ren Wan (Zhong et al., 2013).

Xiao'er Qixingcha is a patent pediatric medicine widely used for various ailments, especially dyspepsia and constipation, which largest manufacturer is Guangzhou Wanglaoji Pharmaceutical Co.Ltd. The sales figures of which reached RMB 198 million in 2009, and exceed 300 million in 2013. In present research, we firstly examined the prokinetic and laxative effects of EXQ, and evaluated its acute toxicity.

In normal mice, the data revealed that EXQ significantly increased the small intestinal transit rates and improved the fecal output.

Consistently, *in vitro* examination revealed that EXQ efficaciously and dose-dependently increased the contraction rates of ileum smooth muscle. Therefore, both *in vivo* and *in vitro* results confirmed the potent prokinetic and laxative effects of EXQ, and suggested that these effects may due to accelerating spontaneous intestinal movement.

Moreover, our results indicated that EXQ has prokinetic and laxative effects against diphenoxylate-induced constipation in mice. Diphenoxylate, a pethidine derivative, is a well known constipation inducer directly effecting on intestinal smooth muscle. This reagent inhibits intestinal peristalsis, slows gastrointestinal transit time, and improves sphincter tone and resting pressure, thus causing infrequent or difficult defecation (Alaradi and Barkin, 2002, Scarlett, 2004). In addition, diphenoxylate increases intestinal water absorption and reduces the water content in stool (Alaradi and Barkin, 2002, Scarlett, 2004). Therefore, diphenoxylate treatment can induce constipation and develop symptoms mimicking clinical constipation. In the present study, the diphenoxylate group showed evident constipation symptoms, including significantly decreased small intestinal transit rate, fecal number and fecal weight in comparison to normal group. Whereas, EXQ treatment dose-dependently relieved these symptoms. The prokinetic and laxative effects of EXQ against diphenoxylate-induced constipation might base on the promotion of intestinal motility and the reduction of water absorption in colon.

Another experimental model employed was hydropenic constipation induced by water-fasting. After water fasting for 72h with free access to diet, the body weights of mice significantly lessened, the feces became few, small and hard, and the urine was less and turned to yellow when compared with the normal control mice (Shan et al., 2010). These symptoms were similar to those of constipated people whose body fluid is lessened and intestine is hydropenic (Shan et al., 2010). Measurement of small intestinal transit rates demonstrated that EXQ dose-dependently improved the propulsion of charcoal meal of water-fasted mice. Furthermore, the decreased fecal output by water-fasting was counteracted by the three dosages of EXQ, and low dose EXQ in 2.92 g/kg exhibited most prominent effect. It’s possibly due to a combinational effect of EXQ together with the highest water content at this lowest EXQ dosage. However, the prokinetic and laxative effect of EXQ is solid, since that the water-fasted group, given an equal volume of saline alone, did show significant constipation compared with the three EXQ groups. Herewith, it can be safely concluded that EXQ displayed prokinetic and laxative effects in hydropenic condition.

Our results demonstrate that Xiao'er Qixingcha exhibited similar prokinetic and laxative effects as Neosigmine Methylsulfate did. Neosigmine Methylsulfate can strengthen the gastrointestinal movement and has been used commonly as a typical positive drug in the studies seeking for potential prokinetic and laxative agents. The dose of Neosigmine Methylsulfate in this study is 2 mg/kg (i.p.). We choose this dose because that half of mice died within 30 minutes after double doses of Neosigmine Methylsuifate (4 mg/kg, i.p.). In contrast, Xiao'er Qixingcha at the doses of 2.92, 4.38, and 6.75 g/kg showed no evident adverse effects.

The equilibrium between therapeutic effects versus the toxicity of a drug is a vital parameter in assessing its safety (Loomis and Hayes, 1996). Data from acute toxicity evaluation displayed that EXQ, up to 66.6 g/kg (202 times of the clinical dosage), had no adverse effect on the subjected animals, which was manifested by the fact that neither changes of body weight and behavior, nor macroscopic lesion was observed. All these indicated that EXQ was a non-toxic agent, being safe for the oral application

Among the component herbs in Xiao'er Qixingcha, *Fructus oryzae germinatus* and *Fructus crataegi* are the two main contributors to stimulate the appetite, as well as relieve dyspepsia and constipation (Pharmacopoeia of PR China, 2010). Research proved that the aqueous extract of *Fructus crataegi* could excite the intestinal constringency in mice, and facilitate the contractility of isolated rat’s gastric and intestinal smooth muscle strips (Ou et al., 2004, Zhang et al., 2009, Wen et al., 2010, Wu et al., 2014). In which, organic acids are revealed to be the major prokinetic active constituents (Wu and Sun, 2009). Therefore, the prokinetic and laxative effects of EXQ might be due to the therapeutic functions of *Fructus oryzae germinatus* and *Fructus crataegi*. However, the precise mechanism underlying this specific action merited further exploration.

Conclusion

Taken together, the present study revealed that the EXQ has a pronounced prokinetic and laxative activity *in vivo* and *in vitro*, which effect possibly mediated by accelerating smooth muscle contraction of small intestine. Thus, Xiao'er Qixingcha might represent a safe and promising therapeutic candidate for constipation, which precise mechanism deserves further research.

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